Amino Acid Composition of α_{s3}-, α_{s4}-, and α_{s5}-Caseins

Abstract

Isolated α_{s5}-casein (zone 0.86) is converted by treatment with 2-mercaptoethanol into two components with electrophoretic mobilities identical to those of α_{s3}-casein (zone 1.04) and of α_{s4}-casein (zone 1.00). The molar ratios of amino acids of α_{s3}-, α_{s4}-, and α_{s5}-caseins are similar. The data suggest that α_{s5}-casein is a molecule of α_{s3}-casein united to a molecule of α_{s4}-casein by at least one intermolecular disulfide bond. Moreover, a close relationship between α_{s3}-casein and α_{s4}-casein is revealed by the similarity of their amino acid molar ratios.

Introduction

The complexity of bovine casein has been documented by the starch gel electrophoresis work of Wake and Baldwin (18). We report the isolation and amino acid analyses of three minor proteins associated with zones 0.86, 1.00, and 1.04 of the Wake and Baldwin gel system. Recently, Annan and Manson have named these minor proteins α_{s5}-, α_{s4}, and α_{s3}-casein, respectively (1). Working with incompletely purified fractions, Annan and Manson have reported 1.03% P for an α_{s3}- and α_{s4}-casein mixture. They found tyrosine and leucine as C-terminal amino acids in the same mixture. We adopt the new nomenclature proposed by Annan and Manson (1) for this report.

Materials and Methods

Whole casein. Casein was isoelectrically precipitated from skimmilk of a cow homozygous for α_{s1}-casein A, β-casein A, and κ-casein A by the method of Thompson and Kiddy (16).

Minor protein, α_{s5}-casein (zone 0.86). A fraction rich in α_{s5}-casein was obtained from a precipitate formed by addition of ammonium acetate to a 50% ethanol solution of α_s-casein by the procedure of Thompson and Kiddy (16). A turbid 2% solution of this precipitate in 25 to 30 ml of 0.01 M imidazole/HCl, 3.3 M urea, pH 7.0 buffer was applied to a 2 × 30 cm DEAE-cellulose column at room temperature. A NaCl gradient, 0.1 M to 0.3 M in 1.8 liters of buffer, was passed through the column at 60 ml/hr. The large eluted peak was cut into fractions. After dialysis and lyophilization each fraction was examined with alkaline polyacrylamide gel electrophoresis for enrichment of α_{s5}-casein. Fractions rich in α_{s5}-casein were pooled from several chromatographic separations and used for preparative polyacrylamide gel electrophoresis.

Minor proteins, α_{s3}- and α_{s4}-caseins (zones 1.04 and 1.00). Essentially the same procedure was followed described for α_{s5}-casein. The critical departure was with 1% mercaptoethanol in the buffer for column chromatography with DEAE-cellulose. α_{s3}-Casein and α_{s4}-casein were incompletely resolved but were free of other proteins. The two minor proteins were further resolved by rechromatography.

Preparative polyacrylamide gel electrophoresis. The E-C Apparatus Company[1] preparative cell was used with the Peterson gel system of 7% Cyanogum, Tris-EDTA-borate pH 9.2 buffer, and 4.5 M urea (10). About 100 mg of the α_{s5}-casein-rich fraction from DEAE-cellulose chromatography was dissolved in 0.5 ml of buffer made 6 M in urea. The solution was applied to a single large slot in the gel slab. After electrophoresis at 300 v for 5 hr, a guide strip was removed from a side parallel to the direction of migration. The strip was stained with Amido Black to locate protein. With the stained strip as a guide, the α_{s5}-casein (zone 0.86) band was excised from the remaining unstained gel slab. The protein was extracted from the horizontally excised strip by electrophoresis performed on the strip held in a dialysis sac filled with pH 9.2 buffer. The ends of the sac were placed in appropriate electrode chambers and a voltage (ca 100 v) was applied to cause the protein to migrate into the buffer at the anode end of the dialysis sac. α_{s5}-Casein was recovered by dialysis of the anodic solution against water followed by lyophilization. Purity of all preparations was verified by alkaline polyacrylamide gel electrophoresis according to Peterson (10). We also found that casein bands in gel slabs can

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[1] It is not implied that the U.S. Department of Agriculture recommends the company mentioned or its products to the possible exclusion of others in the same business.

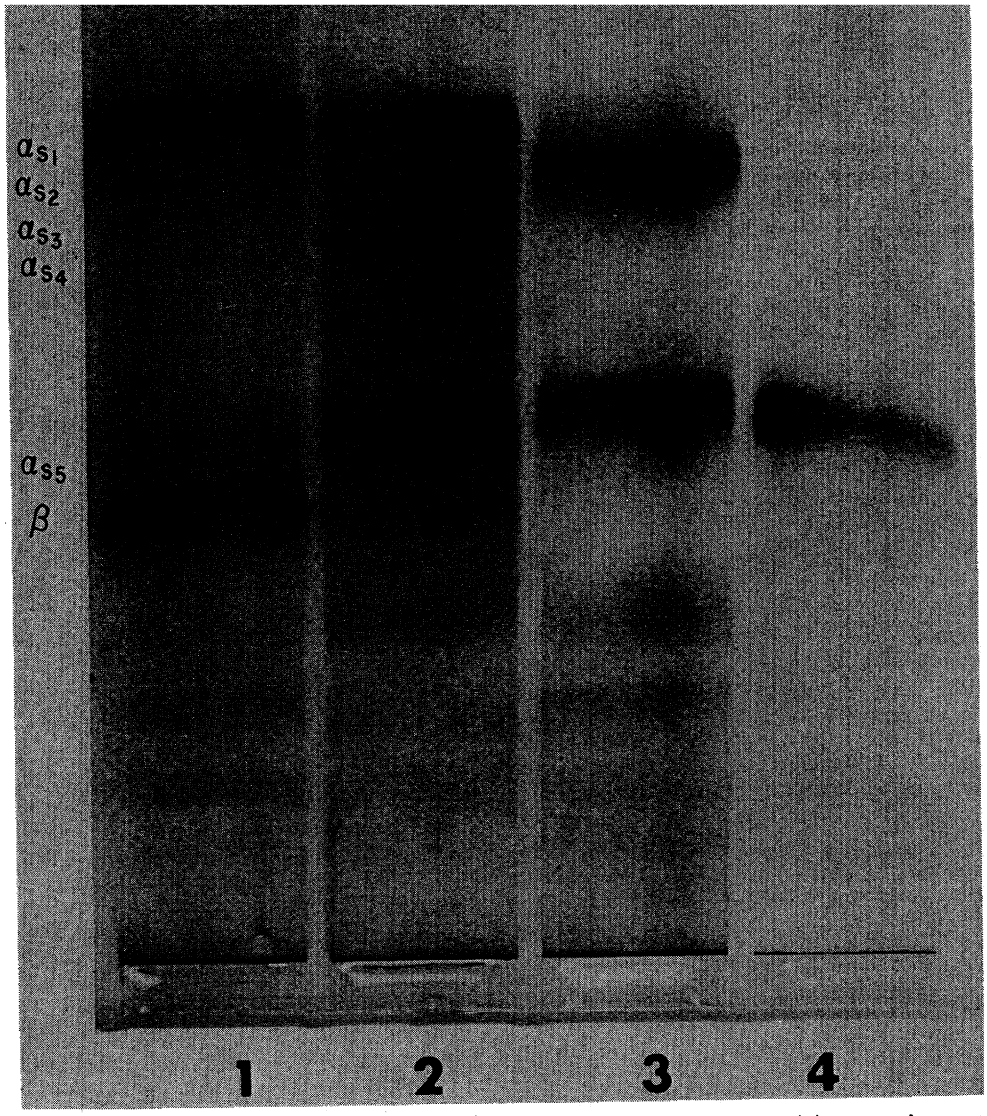

FIG. 1. Alkaline polyacrylamide gel electrophoresis of (1) whole casein, (2) ammonium acetate precipitate of α_s-complex, (3) α_{s5}-casein-rich DEAE-cellulose column chromatography fraction, and (4) α_{s5}-casein from preparative gel electrophoresis. Tris-EDTA-borate buffer at pH 9.2; 6.5% Cyanogum, 4.5 M urea gel.

be precipitated *in situ* with 7% acetic acid. The bands of precipitate can be excised directly. The protein can be recovered by electrophoresis in pH 9.2 buffer, which dissolves the precipitate.[2]

2 These preparations may become contaminated with soluble, nondiffusible polyacrylamide. To increase purity one can precipitate the protein with acid, centrifuge, redissolve at pH 7, dialyze, and lyophilize the aqueous solution to recover the protein.

Amino acid analysis. Hydrolysates were prepared using redistilled 6 N HCl in sealed, evacuated tubes. Hydrolysis was at 110 C for 24, 48, and 72 hr periods in triplicate. The hydrolysates were analyzed for amino acids by the procedure of Piez and Morris (13).

Sugar analyses. The following procedures were used: 1. For hexose, Winzler (20); 2. for hexosamine, Boas (3); 3. for pentose, Dische (5); 4. for hexuronic acid, Dische (4); 5. for sialic acid, Warren (19).

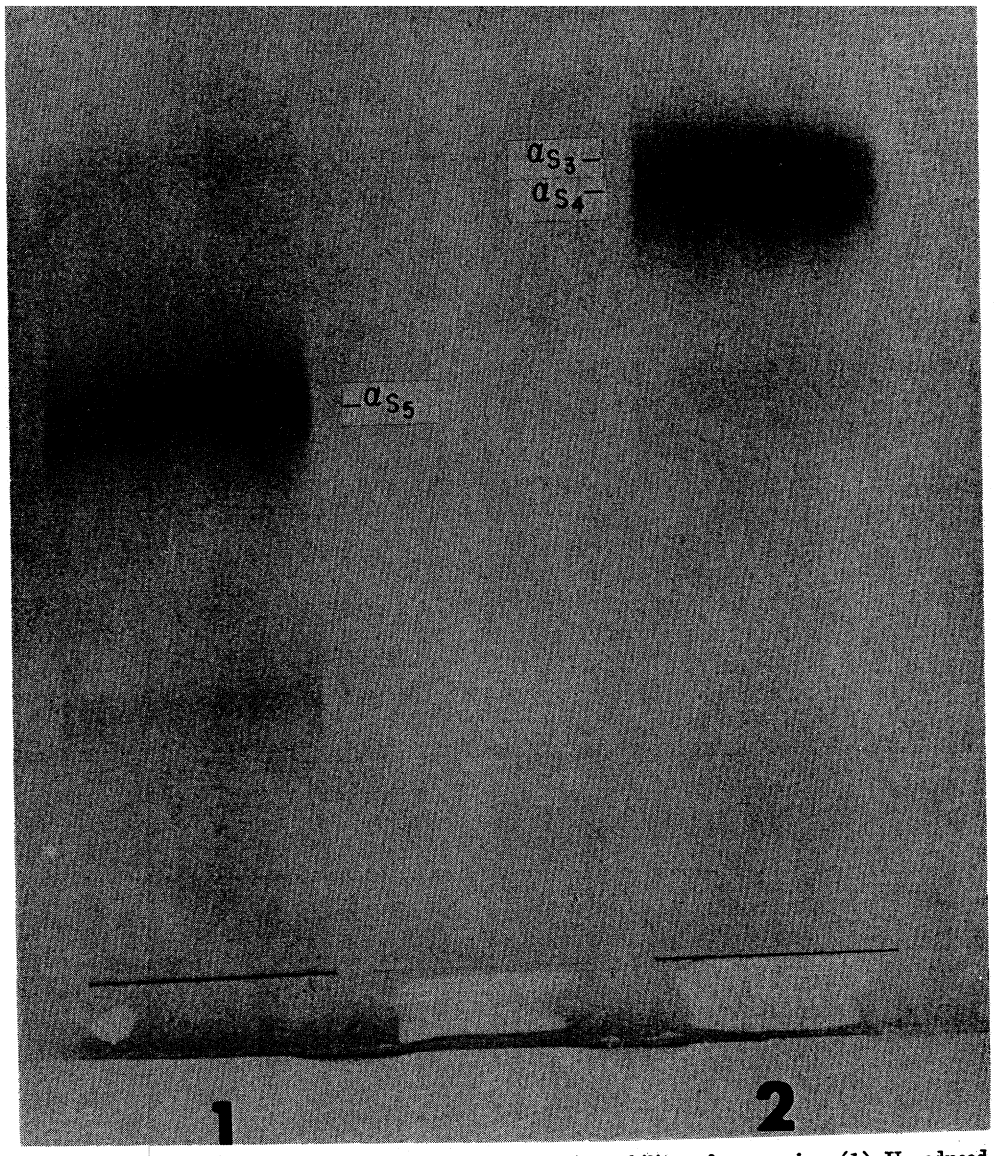

FIG. 2. Effect of 2-mercaptoethanol on electrophoretic mobility of α_{s5}-casein. (1) Unreduced α_{s5}-casein and (2) 1% 2-mercaptoethanol reduced α_{s5}-casein. Tris-EDTA-borate buffer at pH 9.2; 6.5% Cyanogum, 4.5 M urea gel.

Results

The isolation of α_{s5}-casein (zone 0.86) was monitored by alkaline polyacrylamide gel electrophoresis at pH 9.2 (Fig. 1). Preparative gel electrophoresis produced an α_{s5}-casein free of α_{s1}-casein and of β-casein. When α_{s5}-casein (zone 0.86) was reduced with mercaptoethanol, two bands of greater mobility resulted after alkaline polyacrylamide gel electrophoresis (Fig. 2). These bands have mobilities equal to those of α_{s3}- and α_{s4}-caseins (zones 1.04 and 1.00). This behavior is similar to the intensification of α_{s3}- and α_{s4}-caseins and the disappearance of α_{s5}-casein when reduced whole casein is subjected to alkaline gel electrophoresis (Fig. 3).

The elution profiles for DEAE-cellulose column chromatography of α_{s3}- and α_{s4}-caseins (zones 1.04 and 1.00) are reproduced in Figure 4. A good separation of α_{s3}-casein from α_{s4}-

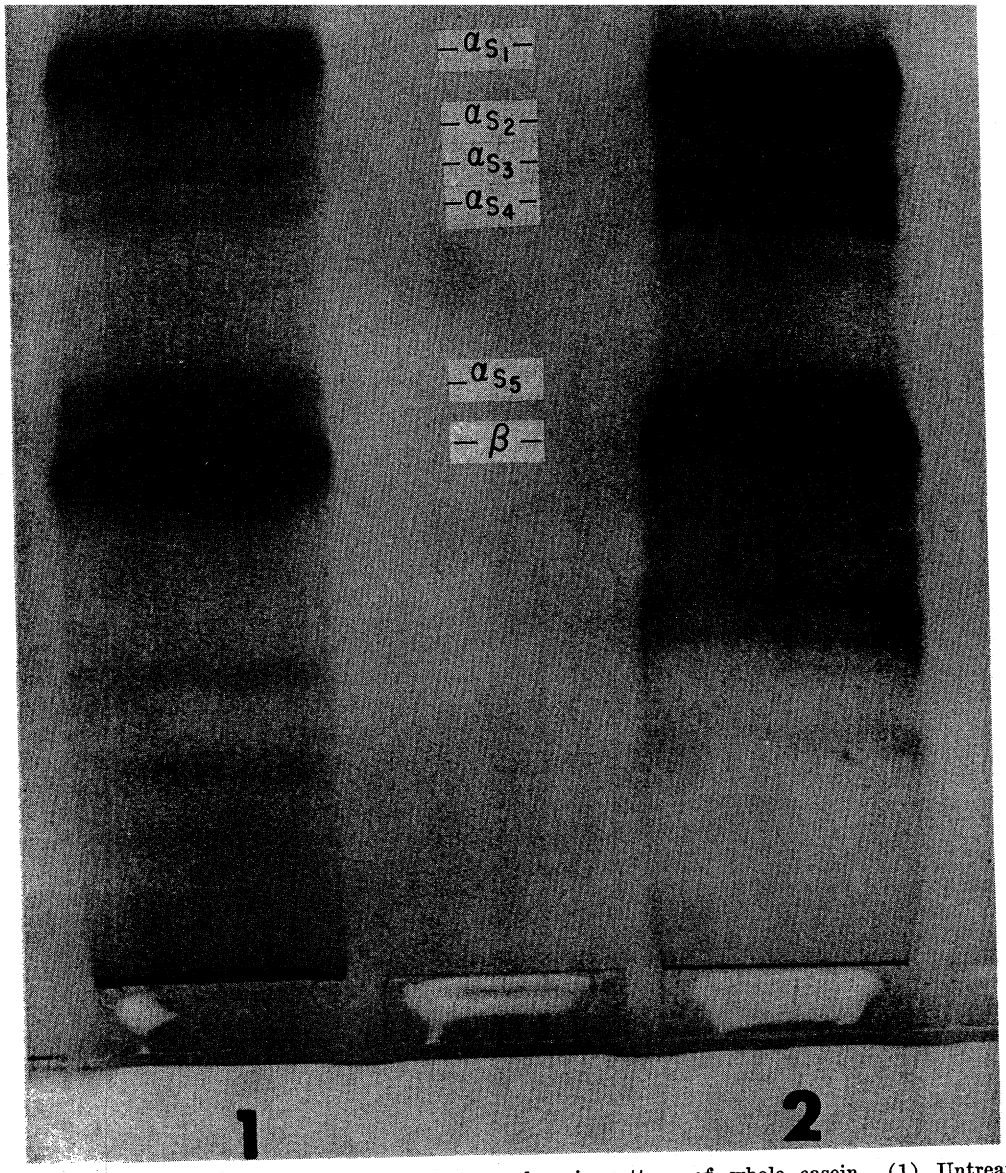

FIG. 3. Effect of 2-mercaptoethanol on electrophoresis pattern of whole casein. (1) Untreated whole casein, (2) 1% 2-mercaptoethanol treated whole casein showing loss of α_{s5}-casein and intensification of α_{s3}- and α_{s4}-casein bands. Tris-EDTA-borate buffer at pH 9.2; 6.5% Cyanogum, 4.5 M urea gel.

casein was obtained, as judged by alkaline gel electrophoresis (Fig. 5).

The results from amino acid analyses of the α_{s3}-, α_{s4}-, and α_{s5}-caseins are in Table 1. The presence of cystine or cysteine in α_{s5}-casein was confirmed by the recovery of cysteic acid from a performic acid oxidation product. The molar ratios of α_{s3}- and α_{s4}-caseins were calculated on the basis of two disulfide bonds per molecule of α_{s5}-casein.

No evidence for the presence of hexose, pentose, hexosamine, hexuronic acid, or sialic acid was found.

Discussion

The amino acid composition of α_{s5}-casein was determined (8) before α_{s3}-casein and α_{s4}-casein were isolated. At that time we did not

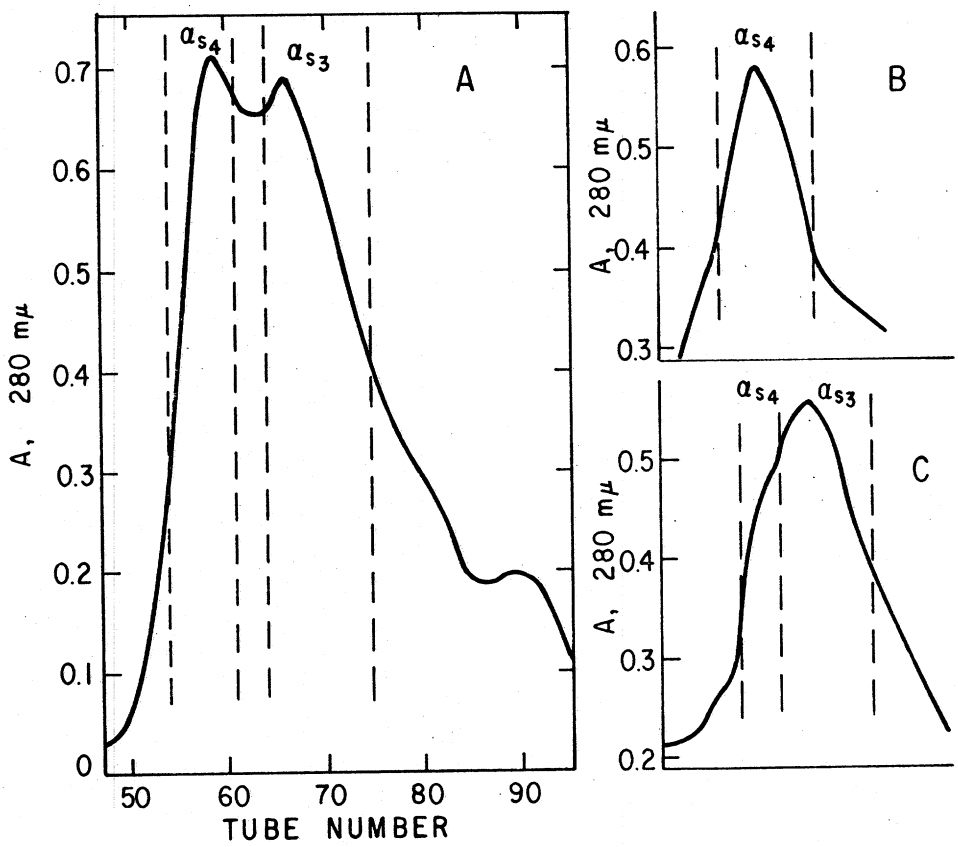

FIG. 4. Elution profile for DEAE-cellulose column chromatography of α_{s3}- and α_{s4}-caseins. One-half gram charge, 0.01 M imidazole/HCl, 3.3 M NaCl gradient. Inserts B and C depict rechromatography of Peaks I (α_{s4}-casein) and II (α_{s3}-casein) in diagram A, respectively.

recognize that the reduction products of α_{s5}-casein migrated the same way as did α_{s3}-casein and α_{s4}-casein during polyacrylamide gel electrophoresis. The slight smearing of the gel pattern in Figure 2 obscures the two bands barely discernible for reduced α_{s5}-casein. We believe that this smearing may reflect partial alteration of the protein by free radical reaction between polyacrylamide and tryptophan residues of α_{s5}-casein (11). Such reaction could have occurred during preparative polyacrylamide gel electrophoresis of α_{s5}-casein.

When the amino acid compositions of α_{s3}-casein and α_{s4}-casein were examined, two unexpected relationships became apparent. The first was that α_{s3}-casein and α_{s4}-casein may be nearly identical, since they have nearly identical amino acid composition. Their difference in electrophoretic mobility may be due to a difference in the number of phosphate groups or a difference in lysine content, or both. The second relationship was that the amino acid composition of α_{s5}-casein is similar to that of α_{s3}-casein and of α_{s4}-casein.

From our experience, α_{s3}-casein and α_{s4}-casein yield bands after alkaline polyacrylamide gel electrophoresis of equal intensity. Moreover, when whole casein is reduced the α_{s5}-casein band disappears and the bands for α_{s3}-casein and α_{s4}-casein intensify equally (Fig. 3). Thus, these latter proteins may occur in casein in an equimolar ratio. In addition, the similarity of amino acid composition of α_{s3}-casein, of α_{s4}-casein and of α_{s5}-casein can be explained by concluding that α_{s5}-casein is composed of one molecule of α_{s3}-casein linked through at least one disulfide bond to one molecule of α_{s4}-casein.

Annan and Manson (1) have calculated a molecular weight of 14,000 for α_{s3}-casein and for α_{s4}-casein. This tentative figure was based on the assignment of different C-terminal amino acids, leucine and tyrosine, for these proteins. Ribadeau-Dumas (14) has reported

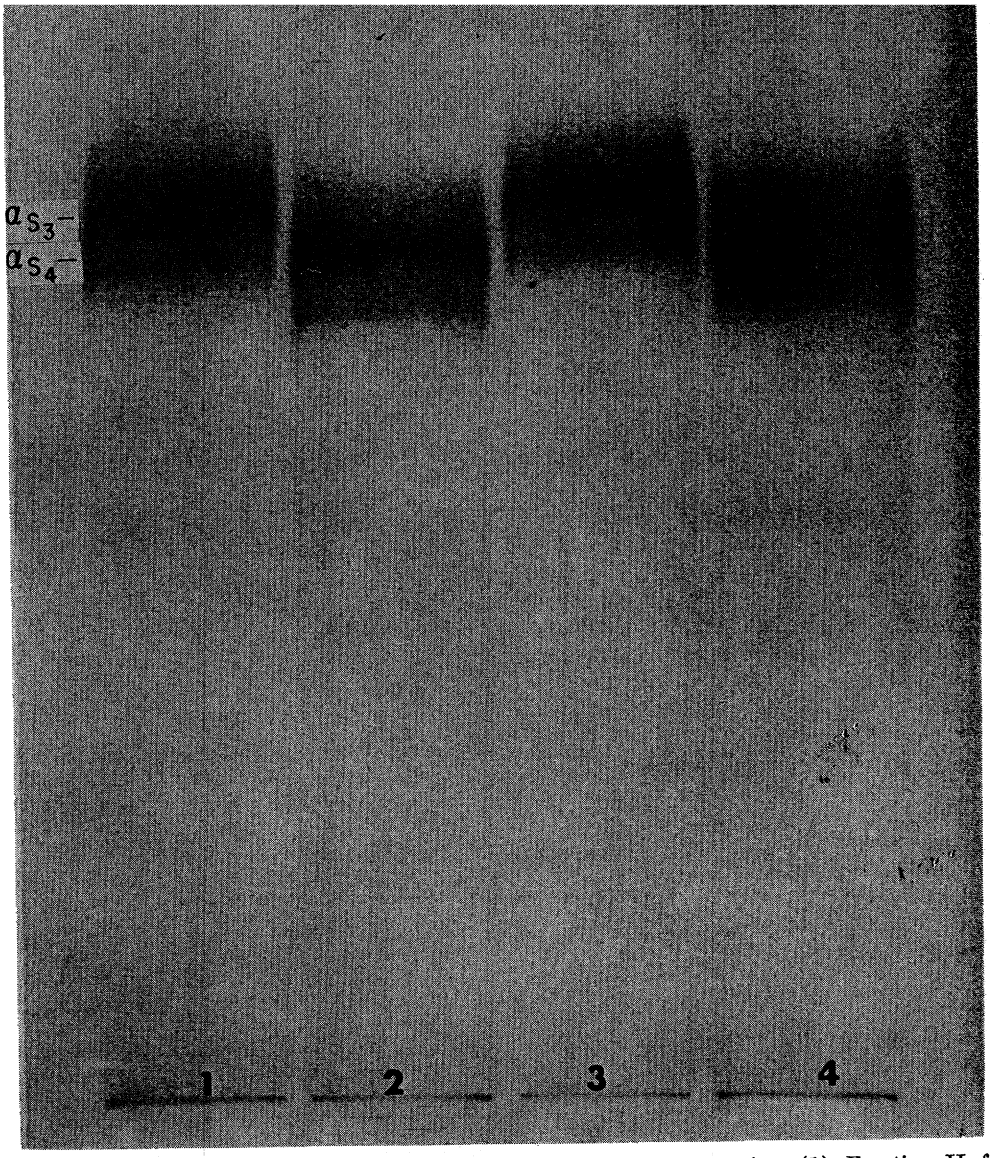

FIG. 5. Alkaline polyacrylamide gel electrophoresis of α_{s3}- and α_{s4}-caseins. (1) Fraction II from Figure 4, (2) Fraction I, (3) Fraction II after rechromatography (α_{s3}-casein), (4) Fraction I after rechromatography (α_{s4}-casein). Tris-EDTA-borate buffer at pH 9.2; 6.5% Cyanogum, 4.5 M urea gel.

a -leu-tyr-OH sequence for both α_{s3}-casein and α_{s4}-casein, calculated from unpublished work. Since we have found that the amino acid composition of α_{s3}-casein and of α_{s4}-casein is similar, we believe the -leu-tyr-OH sequence to be correct.

The molar ratios of amino acids for α_{s3}-, α_{s4}-, and α_{s5}-caseins in Tables 1 and 2 were calculated from the assumption that one mole of -leu-tyr-OH obtains per ca 60,000 g of α_{s5}-casein (1). These ratios yield molecular weights of 67,500 for α_{s5}-casein, and 33,700 for both α_{s3}-casein and α_{s4}-casein. These values are tentative.

Nothing is known about the biological function of α_{s3}-, α_{s4}-, and α_{s5}-casein. If they have no important role in micelle structure, they may prove to have a role in casein biosynthesis, an area that deserves more research attention. Possibly these minor proteins are phos-

TABLE 1. Amino acid molar ratios for α_{s3}-, α_{s4}-, and α_{s5}-caseins.

	α_{s3}-Casein[a]	α_{s4}-Casein[a]	α_{s5}-Casein[a]
Asp	25.7	25.0	52.4
Thr[b]	20.2	19.6	38.3
Ser[b]	22.6	20.8	37.6
Glu	56.3	55.7	111.8
Pro	15.5	15.2	29.7
Gly	4.0	3.9	8.7
Ala	11.1	11.3	24.3
Cys/2[c]	1.4	1.5	3.7
Val	18.8	19.5	36.8
Met	5.5	5.0	8.8
Ile	15.1	14.9	29.3
Leu	17.4	17.9	36.8
Tyr	13.9	14.0	27.5
Phe	8.1	8.5	17.5
Lys	29.7	32.1	62.5
His	5.3	5.5	10.2
Arg	7.4	8.3	17.1
Try[d]			4
Cysteic acid[e]			3.9

[a] Average of analyses of the hydrolysates at 24, 48 and 72 hr.

[b] Linearly extrapolated to zero time.

[c] Uncorrected for decomposition. R. Jenness examined enriched fractions of α_{s3}-casein and of α_{s5}-casein and found sulfhydryl groups present sufficient to give one disulfide per ca 34,000 Mole Wt.

[d] Carried out according to Spies and Chambers (15).

[e] Value from performic acid oxidized α_{s5}-casein.

phorylated by the same process that incorporates specifically different numbers of phosphate groups into the major casein proteins.

We would like to comment on the nomenclature of the minor proteins of the α_s-complex of bovine casein. Thompson et al. (17) tentatively classified zone 1.04 as α_{s2}-casein and zone 1.00 as α_{s3}-casein. Annan and Manson (1) have subsequently found an additional minor protein that migrates just behind α_{s1}-casein during alkaline gel electrophoresis. This component was not considered in the nomenclature scheme of Thompson et al. (17). Since this component is found in many, if not all, caseins, we recommend that the nomenclature scheme of Annan and Manson (1), the most complete scheme offered to data for the α_s-caseins, be adopted. For this reason we now refer to zone 1.00 as α_{s4}-casein, to zone 1.04 as α_{s3}-casein, and to zone 0.86 as α_{s5}-casein.

At present the nomenclature of the minor proteins of the α_s-complex is uncomplicated by genetic variation. No polymorphism of α_{s3}- and α_{s4}-caseins has yet been observed in Western breeds of cattle. However, Aschaffenburg et al. (2) have shown that α_{s3}- and α_{s4}-caseins are polymorphic and therefore probably genetically variable in zebu casein. Of related interest is the report of Michalak (9) that the caseins of some Red Danish cattle give no α_{s3}- and α_{s4}-casein bands (zones 1.04 and 1.00) after starch gel electrophoresis. Thus, these proteins may be either absent from these caseins or migrating quite differently (possibly in the κ-region).

TABLE 2. Amino acid residue composition of some proteins of bovine casein.

	α_{s1}-B (6)	β-A^2 (12)	κ-A (21)	γ-A^2 (7)	α_{s4}-[a] (Tentative)
Asp	18	9	12	9	25
Thr	6	9	14	10	20
Ser	17	15	12/13	13	21
Glu	46	39	27	39	56
Pro	20	34	20	41	15
Gly	11	5	3	5	4
Ala	11	5	13/14	6	11
Val	13	18	10/11	20	19/20
Cys/2	0	0	2	0	2
Met	6	6	2	7	5
Ile	13	10	11	8	15
Leu	20	21	8	23	18
Tyr	12	4	8	5	14
Phe	10	9	4	11	8/9
Trp	3	1	1	1	2
Lys	17	11	9	12	32
His	6	5	3	6	5
Arg	7	4	5	3	8
P	11	5	1	1	10[b]
	28,820	23,590	18,780	24,850	33,700

[a] Residue composition based on one disulfide per molecule of α_{s4}-casein.

[b] Value based on 1.03% P reported for α_{s3}- and α_{s4}-caseins by Annan and Manson (1). We obtained 0.72–1.04% P for enriched fractions of α_{s4}-casein and of α_{s5}-casein. These analyses were kindly performed by S. B. Jones and G. Mychaluk on material prepared by a Sephadex chromatographic procedure not yet published.

[c] Based on composition shown, exclusive of any carbohydrate content.

Acknowledgments

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